SUPPLEMENTARY FIGURES

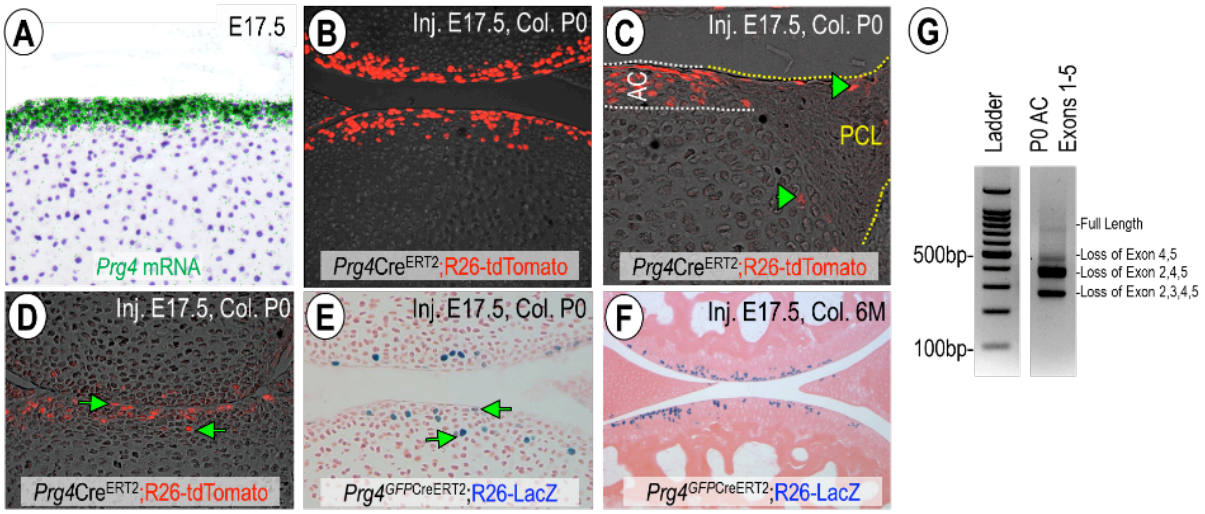

Fig. S1: *Prg4*+ cells are present throughout incipient articular cartilage at P0 and various *Prg4* variants are expressed. **(A)** At E17.5, in situ hybridization for *Prg4* demonstrates that transcripts are present throughout the entire thickness of the prospective articular cartilage. **(B)** After tamoxifen induction of *Prg4*-CE/R26-tdTomato mice at E17.5, *Prg4*+ cells are found throughout the articular layers at cartilage at birth. **(C)** *Prg4*+ are less numerous within the posterior cruciate ligament (PCL) and enthesis (arrowheads) compared to the adjacent articular cartilage (AC). **(D-F)** Commercially available knock-in *Prg4*^{GFPCreERt2} mice used in previous studies (Kozhemyakina et al., 2015) were mated with *R26-tdTomato* or *R26-LacZ* mice to repeat those studies. Mice were induced with tamoxifen once at E17.5 and examined at P0. In line with our results in (**B**), we observed that *Prg4*+ cells are present throughout the cartilage layers (**D**, **E**, arrows) and are not limited to the most superficial layer as reported previously (Kozhemyakina et al., 2015). **(F)** Monitoring of companion mice at 6 months of age shows that *LacZ*-positive cells remain present throughout all layers. **(G)** Multiple splice variants of *Prg4* are expressed in P0 tibial cartilage lacking combinations of exons 2-5.

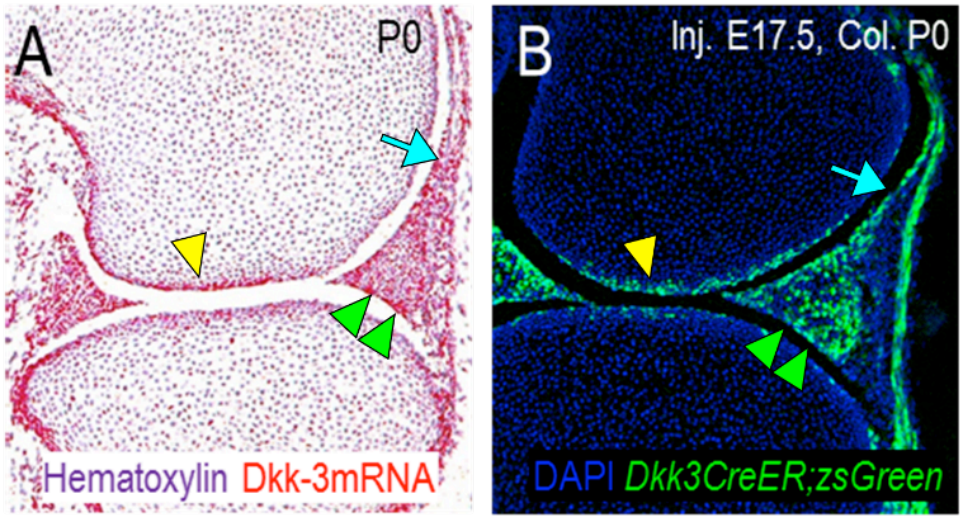

Fig. S2: *Dkk3* expression characterizes multiple early joint cells. **(A)** At P0, *Dkk3* mRNA expression is found in the articular cartilage (arrowhead), synovial lining (arrow) and meniscus (double arrowhead) of mouse knee joints. **(B)** Reporter-positive cells in *Dkk3-CE;R26-zsGreen* knees in mice injected with tamoxifen at E17.5 match the mRNA expression patterns and are present in articular cartilage (arrowhead), synovial lining (arrow) and meniscus (double arrowhead).

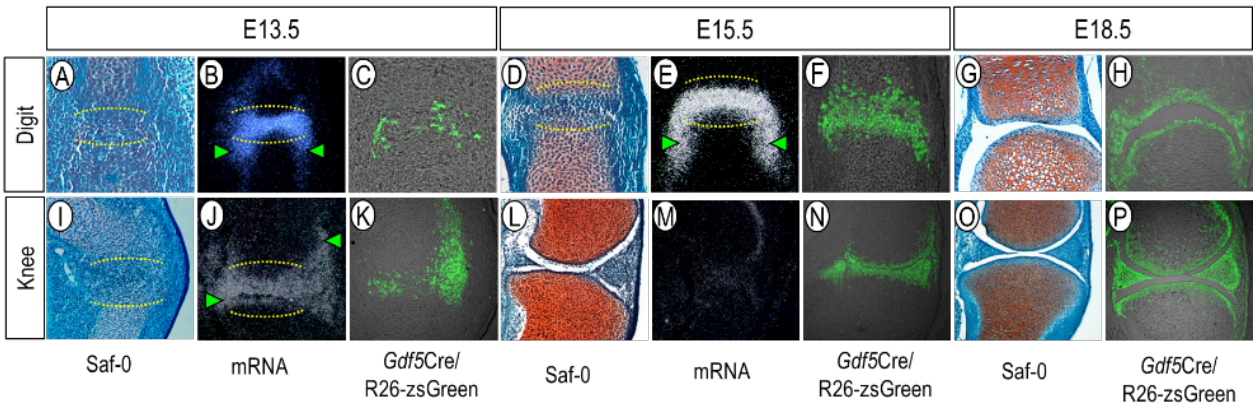

Fig. S3: *Gdf5*+ cells within and flanking the interzone give rise to most/all joint tissues over time. At E13.5 **(A-C, I-K)** and E15.5 **(D-F, L-N)**, *Gdf5*-expressing cells are found within (yellow dotted lines) and flanking (green arrowheads) the interzone tissue in knee and digit joints, as reflected by *Gdf5*Cre/R26-zsGreen expression **(C, F, K, N)**. By E18.5 (**G,H, O,P**), joints have fully cavitated, and *Gdf5*Cre/R26-zsGreen expression reveals that *Gdf5*+ cells have given rise to multiple joint tissues.

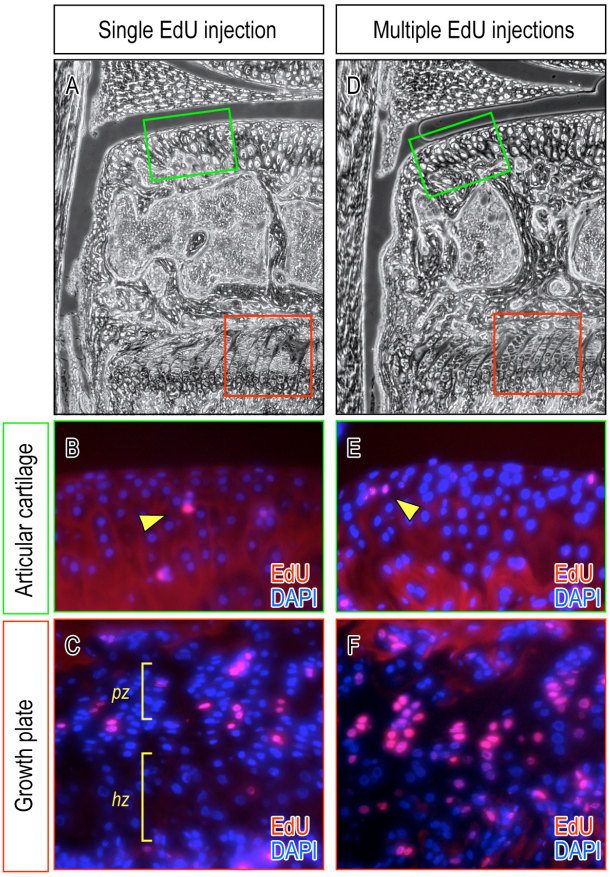

Fig. S4: Proliferation of superficial and non-superficial joint cells is low at juvenile stages. P28 mice were injected with EdU (50 mg/Kg/day, IP) once (**A-C**) or 10 times over 10 consecutive days (**D-F**), and their knees were processed for serial sectioning and detection of incorporated EdU, using an Invitrogen kit (C10337). The labeling conditions and mouse age were exactly as those used by Kozhemyakina et al., 2015. Note that after a single or multiple EdU injections, the number of labeled cells in tibial articular cartilage is minimal (**B** and **E**, arrowhead), amounting to 3 to 5% of the total cell population. Green boxed areas in **A** and **D** are shown at higher magnification in **B** and **E**. In comparison, numerous EdU-labeled cells are observable in the proliferative zone (*pz*) of underlying growth plate after 1 injection (**C**) and in both the proliferative zone and hypertrophic zone (*hz*) after 10 daily injections (**F**), attesting to the fact that the labeling protocol was effective in both cases. Red boxed areas in **A** and **D** are shown at higher magnification in **C** and **F**.

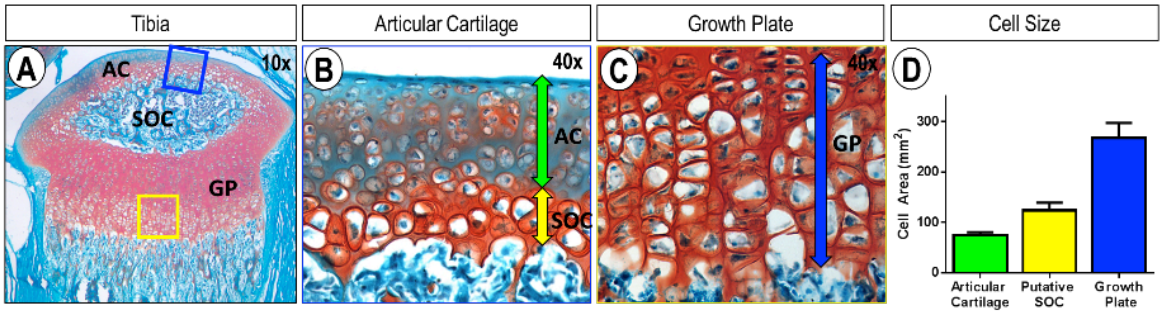

Fig. S5: Chondrocyte size and diameter vary by location and function. **(A-C)** Safranin-O/Fast Green stained sections of mouse proximal tibia at P14 showing the articular cartilage (AC), secondary ossification center (SOC) and underlying growth plate (GP). Squared areas are shown enlarged in (**B**) and (**C**). **(B)** Chondrocytes in the articular cartilage are smaller in size than those in the underlying SOC, the latter identified also by *Matrillin-1* expression. **(C)** The largest chondrocytes are found within the hypertrophic cells of the growth plate along the chondro-osseous border. (**D**) Average two-dimensional size of largest individual cells measured in 12 µm-thick tissue sections by ImageJ software. Data are shown as mean ± S.D.

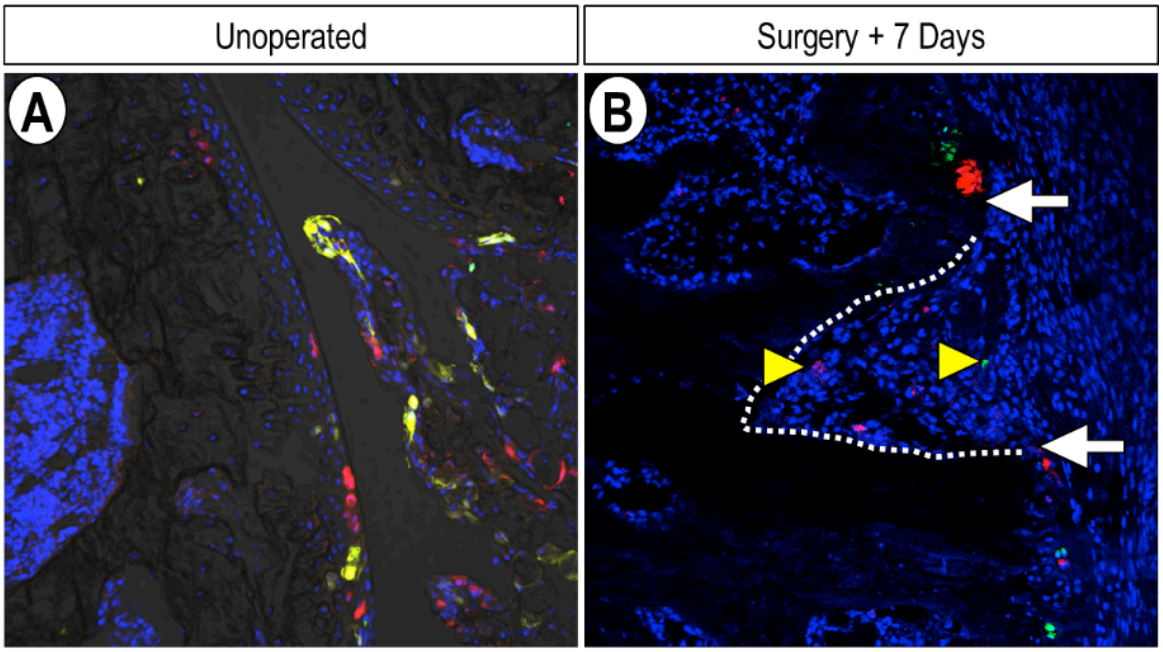

Fig. S6: *Gdf5*+ cells within adult articular cartilage do not undergo clonal expansion in response to acute cartilage injury. (A) At 2 months, uniquely colored clones of *Gdf5Cre/R26-Confetti* are found throughout the articular surface of femur in un-operated knees. **(B)** By day 7 after acute femoral cartilage injury, *R26-Confetti* labeled cells are found within the defect (dashed line, arrowheads), but there is no evidence that uniquely labeled cells within adjacent cartilage proliferate to fill the defect site in such acute injury setting (arrows).

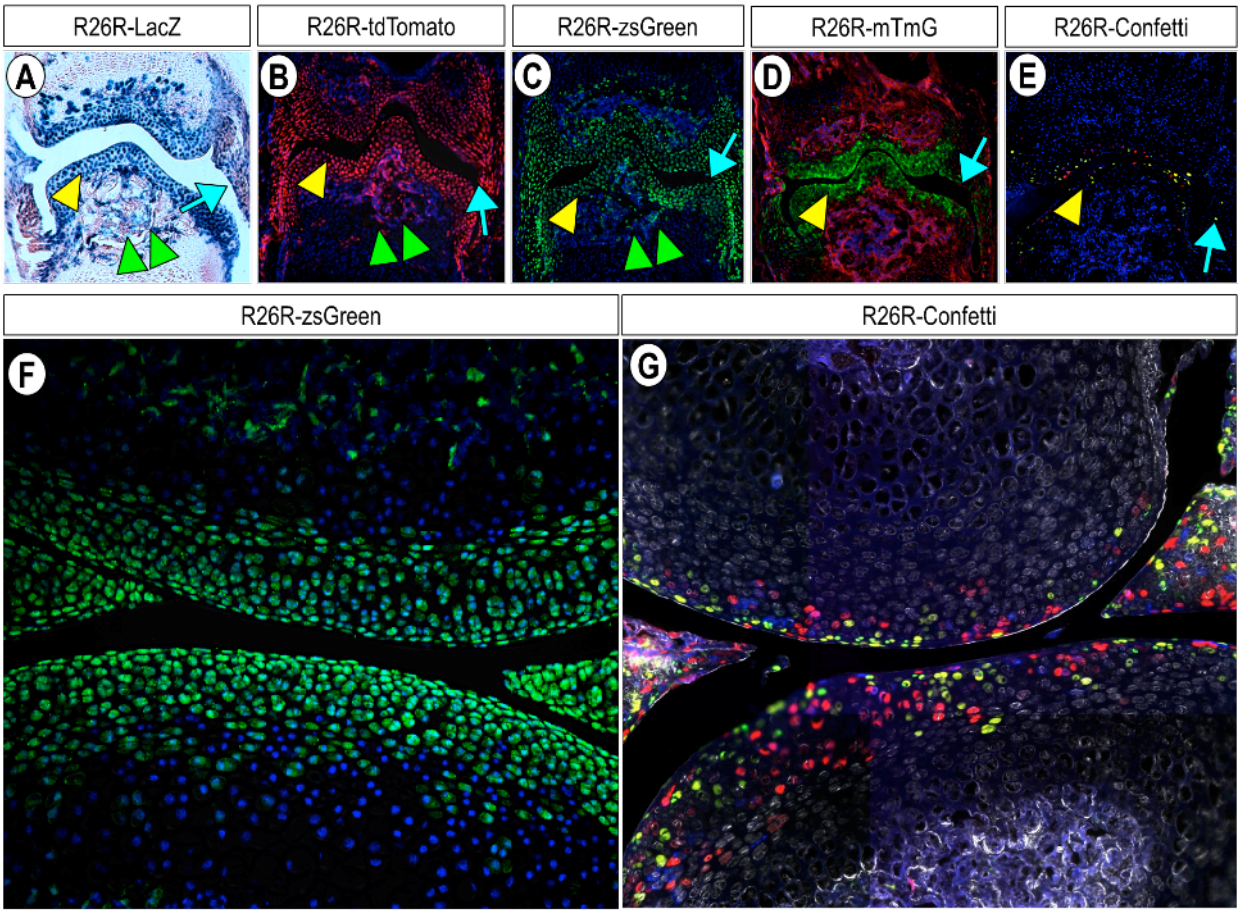

Fig. S7: Genetic labeling efficiency of *Gdf5*+ cells in limb joint tissues varies among common R26-reporters lines. (A-C) *Gdf5*+ cells are present in the articular cartilage (yellow arrowhead), synovial lining (blue arrow) and secondary ossification center (green double arrowheads) of (**A**) *Gdf5Cre/R26-LacZ*, (**B**) *Gdf5Cre/R26-tdTomato* and (**C**) *Gdf5Cre/R26-zsGreen* mice in P14 metacarpophalangeal joints. **(D-E)** Labeling efficiency and/or specificity are different in (**D**) *Gdf5Cre/R26-mTmG* and (**E**) *Gdf5Cre/R26-Confetti* mice at the same stage, where reporter expression is limited to articular cartilage (yellow arrowhead), synovial lining only (blue arrow). **(F-G)** Similar labeling patterns are seen in P14 knees of (**F**) *Gdf5Cre/R26-zsGreen* mice and (**G**) *Gdf5Cre/R26-Confetti* mice.